VII. REFERENCES

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VIII. REPORT FORMAT

The final report will provide the following information.

- Sponsor identification
- Quality Assurance statement
- Statement of GLP Compliance with signature of study director
- Test article identification
- Type of assay and protocol number
- Dates of study initiation and completion
- Identity of the study director and laboratory supervisor
- Methods
- Evaluation criteria
- Interpretation of results
- Conclusions
- References
- Test results presented in tabular form
- Copy of the signed protocol and all amendments
- Historical control data
- Sponsor-provided test article information and analytical data

IX. CHANGES OR REVISIONS

Any changes or revisions of this approved protocol will be documented, signed by the Study Director, dated, and maintained with this protocol.

X. RECORDS TO BE MAINTAINED

All raw data, documentation, records, protocols, and final reports generated as a result of this study will be archived.

DEFINITION OF CHROMOSOME ABERRATIONS FOR GIEMSA STAINED CELLS

NOT COMPUTED

TG	Chromatid Gap ("tid gap"):	An achromatic (unstained) region in one chromatid, the size of which is equal to or smaller than the width of a chromatid. These are noted but not usually included in final totals of aberrations as they may not all be true breaks.
SG	Chromosome Gap: ("isochromatid gap, IG"):	Same as chromatid gap but at the same locus in both sister chromatids.
UC	Uncoiled Chromosome:	Failure of chromatin packing. Probably not a true aberration.
PP	Polyploid Cell:	A cell containing multiple copies of the haploid number (n) of chromosomes.
E	Endoreduplication:	4n cell in which separation of chromosome pairs has failed.

SIMPLE

TB	Chromatid Break:	An achromatic region in one chromatid, larger than the width of a chromatid. The associated fragment may be partially or completely displaced.
SB	Chromosome Break:	Chromosome has a clear break, forming an abnormal (deleted) chromosome with an acentric fragment that is dislocated. This classification now includes the acentric fragment (AF). The AF was different from the SB only in that it was not apparently related to any specific chromosome.
DM	"Double Minute" Fragment:	These are small double dots, some of which are terminal deletions and some are interstitial deletions and probably small rings. Their origins are not distinguishable.

COMPLEX

ID	Interstitial Deletion:	Length of chromatid "cut out" from midregion of a chromatid resulting in a small fragment or ring lying beside a shortened chromatid or a gap in the chromatid.

TR	Triradial:	An exchange between two chromosomes, or one chromosome and an acentric fragment, which results in a three-armed configuration.
QR	Quadriradial:	As triradial, but resulting in a four-armed configuration.
CR	Complex Rearrangement:	An exchange among more than two chromosomes or fragments which is the result of several breaks.
D	Dicentric:	An exchange between two chromosomes which results in a chromosome with two centromeres. This is often associated with an acentric fragment in which case it is classified as DF.
DF:		Dicentric with fragment.
TC	Tricentric:	An exchange involving three chromosomes and resulting in a chromosome with three centromeres. Often associated with two to three AF. Such exchanges can involve many chromosomes and are named as follows:
QC	Quadricentric:	Four centromeres, up to four AF
PC	Pentacentric:	Five centromeres, up to five AF
HC	Hexacentric:	Six centromeres, up to six AF
R	Ring:	A chromosome which forms a circle containing a centromere. This is often associated with an acentric fragment in which case it is classed as RF.
RC	Ring Chromatid:	Single chromatid ring (acentric).
RF:		Ring with associated acentric fragment.
CI	Chromosome Intrachange:	Exchange within a chromosome, (e.g., a ring that does not include the entire chromosome).
T	Translocation:	Obvious transfer of material between two chromosomes resulting in two abnormal chromosomes. When identifiable, scored as "T" not "2Ab."

AB

Abnormal monocentric chromosome. This is a chromosome whose morphology is abnormal for the karyotype, and often the result of a translocation, pericentric inversion, etc. Classification used if abnormality cannot be ascribed to; e.g., a reciprocal translocation.

<u>OTHER</u>

GT

Greater than 10 aberrations: A cell which contains more than 10 aberrations. A heavily damaged cell should be analyzed to identify the types of aberrations and may not actually have >10, (e.g., multiple fragments such as those found associated with a tricentric).

E197

MODERN BIOELECTRICITY

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Preface

It has been almost 25 years since I was introduced to bioelectricity by my teacher, Robert Becker. The subject was then in its infancy and had no natural constituency because it did not fit easily within any of the orthodox scientific pigeonholes. In physiology, electricity usually meant action potentials, within engineering it related to microwave heating, in physics and medicine it was associated with X-rays and radiotherapy treatment of cancer, and in chemistry it was linked to electrode reactions. Against this backdrop, two important themes emerged. What is the nature of the system or process that controls the living organism? Some might hold it to the finger of God, and declare its inherent mechanics to be unknowable. The other extreme involves focusing on molecular minutiae in the belief that life can be defined at that level. Modern bioelectricity is a middle-of-the-road approach which began with a crystallizing perception that electrical interactions are more fundamental than biochemical reactions, and hence that they perhaps have a greater probability of explaining the physical basis of life and the processes that control and express it. Bioelectricity's other major theme -- environmental electromagnetic pollution -- became important beginning in the early 1970's.

Much has happened during the past two decades, and this book is a monument to that work. As with all new initiatives, many questions have been raised, and previously unrecognized problems have become manifest. But it is the business of science to uncover and solve these problems, and it is precisely this effort, which is taking place on a broad scale across many traditional scientific disciplines, that constitutes the chief development in bioelectricity during the past 20 years. To

17

Behavioral Measures of Electromagnetic Field Effects

ROCHELLE MEDICI

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WHY BEHAVIOR?

There are at least three major reasons for studying the behavioral effects of electromagnetic (EM) fields: First and most importantly, behavioral studies are a sensitive and reliable measure of the functioning of the central nervous system (CNS). Secondly, behavioral studies can validate or invalidate theories about CNS mechanisms of interaction. (Conversely, behavioral studies may lead to the formulation of such theories.) Thirdly, behavioral studies can provide us with sound ideas for practical applications of EM research. Such studies can define both the promise and the limitations of EM fields as a technique for changing or modifying human behavior.

Concern with practical issues of changing human behavior with EM energy has caught the attention of the press and the public in the last two decades. The "zapping" of the United States' Embassy in Moscow in the 1960s led to speculation that very weak pulsed EM fields might lead to dramatic thought disorders or physical illness in the Embassy staff. This focus has detracted from the sure, steady, and unglamorous results of behavioral studies, which have advanced our understanding of how EM fields affect living organisms, and our understanding of the role of the CNS in mediating these effects.

BEHAVIOR AS A SENSITIVE AND RELIABLE MEASURE OF CENTRAL NERVOUS SYSTEM EFFECTS

The major significance of behavioral studies is that they offer a sensitive measure of CNS function. It is possible to vary power, frequency, modulation and duration of exposure of EM fields, and to determine precise, dose-related changes in the behavior of an experimental animal, and thus assess effects on CNS function.

It is a common error to believe that behavioral measures of brain function are merely phenomenological, and that they are less precise, reliable, and real than physiological measures. This is not so. For example, electroencephalograms (EEGs) are in many ways a very gross measure of CNS function. Spectral analyses of EEGs from electrodes implanted deep in the brain of animals exposed to EM fields reveal great limitations in this technique. Questions of sampling adequacy and statistical inference are not easy to resolve. Medical imaging offers a limited picture of brain structure and pathology but, on occasion, behavior can tell us more. For example, the impact of low doses of drugs or EM fields may not permanently alter the brain. Behavioral studies can reveal important, but transient, changes. Studies of biochemical changes *in vitro* leave us with the problem of extrapolating the significance of any observed changes to the living animal.

A lack of knowledge about the science of behavior appears frequently in the nonionizing radiation area. Frequently, researchers regard all behavioral measures as equivalent. But actually there is no more equivalence between an activity measure of behavior and an inter-response time schedule of reinforcement than there is between measuring temperature by putting a hand on a child's forehead and measuring temperature with a precise gauge.

The appropriate model for behavioral studies of EM fields is that of research on low doses of drugs. Techniques for precisely assaying effects of minimal drug doses were developed in the United States in the 1930s. Essentially, these techniques involve training animals to perform simple, measurable behavioral tasks (schedule-controlled behavior, as originally described by B.F. Skinner). Following the training, one can then measure the degree to which defined doses of drugs -- or EM fields -- perturb that behavior. The perturbation of behavior, the measure of neural function, can be precisely measured.

With such techniques, it is possible to precisely study the effect of gradually increasing the power of the EM field, to measure effects of increasing the duration of exposures, to measure changes due to the introduction of modulation in a constant frequency field, and so on. The work of Thomas and his associates (1-7) is an excellent example of this approach. However, as we shall see in the review of the literature that follows, many other studies of EM fields were done with simple-minded and insensitive behavioral techniques.

BEHAVIOR AS A NECESSARY MEASURE OF THE VALIDITY OF THEORIES OF CENTRAL NERVOUS SYSTEM MECHANISMS OF INTERACTION

Behavioral studies provide the ultimate validation of hypotheses about CNS mechanisms. For example, Adey has proposed that since weak EM fields can affect calcium efflux (in *in vitro* chicken brains), present theories about CNS function must be radically changed. He argues that one should adopt a "nonequilibrium viewpoint" in which cells "whisper" to each other so that very low amounts of energy can affect vast arrays of neurons (8). Such theories have great intellectual appeal and fascination, but unless experiments are done to link them directly to changes in behavior, they remain an empty and trivial exercise. Earlier psychobiologists recognized the problem of theories that remain "locked in the mind." The most grandiose theory of neural functioning is useless if it is not anchored in relevant experimentation.

There are other requirements of a robust theory. It is essential that the theory do more than simply restate, in theoretical neurobiological terms, the empirical observations which led to its formulation (i.e., weak EM fields perturb behavior). Good theories should provide hypotheses that generate practical experiments. These new hypotheses must be testable. In the case of the nervous system and EM fields, this means testing at the behavioral level. The recent work of Thomas, et al. (5) is an example of behavioral validation of a neural theory.

Not only are behavioral studies necessary to the validation of neural theories, but they are also a rich source of ideas for the generation of such theories.

PRACTICAL APPLICATIONS OF BEHAVIORAL STUDIES OF ELECTROMAGNETIC FIELD EFFECTS

It has been said that man's egocentric concerns at the time of Copernicus made it difficult for him to accept the notion that the sun rather than the earth is the center of the world. Our egocentric concerns may be drawn to rather sensational notions about the capability of EM fields to change human behavior. These concerns detract from the significance of solid, modest, laboratory studies. It is the implications of these laboratory studies for basic science which is ultimately significant.

It is conceivable that "...specific frequencies might affect different kinds of learning. One frequency might aid in memory retention; another might enhance performance in music, art, or mathematics since these are all very specific talents which involve different brain structures and different kinds of electrical activities" (9). However, the research to determine whether these applications are feasible, practical, or desirable has not been done. We need intensive, appropriate, research at the laboratory level. Epidemiological studies of EM effects reflect some of our concerns with hazard; such studies are often criticized for their lack of precision. However, if more laboratory studies were done, the guiding hypotheses for epidemiological studies could be stated far more definitively and better studies could be designed at the outset.

We need to understand and measure the dose-effect parameters of EM radiation including the effects of power, frequency, duration of exposure, and modulation. Then we may foresee both the limitations and benefits of this research for human application.

HISTORICAL PERSPECTIVES

Despite the fact that pioneering studies in nonionizing radiation were done in the 1960s (10), little relevant research occurred subsequently. Why?

Each new behavioral study that appeared was scrutinized, analyzed, criticized, and challenged by scientists who had been active in earlier EM research and in hazard standard-setting. As one of these scientists put it when he testified at the New York State Public Service Commission Hearings in 1976, "...whenever the claim was that no effect was observed, ... I was not

further interested in digging into the material... I didn't see any motivation to dig very deeply into the statistics whenever the effect was reported null. I felt more motivated to dig into it if there was an effect reported... I think that adequately summarizes my approach to the evaluation..." (11).

Some scientists believed that it was simply impossible for low-level EM fields to affect behavior because the energy was small. Only a new awareness of neuroanatomy and the neural sciences eventually eroded this kind of objection.

There was also a problem from groups with vested interests. The military did not want to hear about possible hazards associated with radar installations. Microwave-oven manufacturers saw a threat to a new and booming business. Money for grants became very limited. At times it seemed that grant money was only available for investigators who were willing to do monolithic studies that used such insensitive biological measures that they were literally guaranteed to show that neither hazard -- nor effects of any kind -- occurred in the presence of weak EM fields. For example, Guy was awarded a multi-million dollar grant to do a long-term study at the University of Washington which used a variant of the open-field test as its only measure of the nervous system and behavior (12). This simplistic and insensitive behavioral measure would be guaranteed to show no effect to almost any kind of weak environmental stimuli. The politics of funding for EM research are discussed elsewhere (13).

In my opinion, another factor that slowed EM behavioral research was a lack of understanding of the science of behavior. Perhaps this is due in part to the inter-disciplinary nature of EM research. Behavioral studies were undertaken by physicists, engineers, veterinarians, physicians, and only occasionally by psychologists or psychobiologists. Elegant, sensitive, schedule-controlled tests of behavior were developed in the United States in the 1930s and have been widely used in toxicology and pharmacology to assess the effects of low doses of drugs. Yet much EM research has focused on insensitive, simplistic tests of behavior (open-field tests, activity tests). Or, ironically, they have focused on replication of Soviet techniques from conditioning studies that date back to about 1910 (e.g., foot withdrawal to shock).

In the 1970s, many EM researchers tended to equate one behavioral test with another. If sensitive, schedule-controlled tests showed effects, it was argued that these were negated by the lack of effects from an experiment in which an insensitive measure such as activity was used. A major thrust of this paper is to demonstrate that behavioral results can only be evaluated in the context of the adequacy or reliability of the specific behavioral measure which is used.

PRESENT STATUS OF ELECTROMAGNETIC BEHAVIORAL STUDIES

There are three major factors that affect experimental outcome (14): (1) the behavioral measure used; (2) whether the field is modulated; (3) whether the field is primarily magnetic or electric. Other variables such as the carrier frequency, intensity, and duration of exposure may also affect the result, but the primary importance of the three listed factors has been convincingly established.

The primary lesson to be learned from earlier reviews of the literature (15-17) holds true today. It may be stated rather simply: Behavioral techniques may be considered on a continuum proceeding from almost no external stimulus control (for example, open field tests and activity measures) to techniques in which the animal is required to respond to demanding elements of the task itself (as in escape or avoidance tasks). At one end of the continuum the behavior is too variable to adequately reflect the effect of a weak nonionizing field. At the other end of the continuum the animal is too preoccupied with the demands of the task to attend to the effect of the imposed fields. In between these two extremes are a variety of relevant schedule-controlled techniques, especially those which are time-based, that are both reliable and sensitive (15-17).

Among schedules of reinforcement (reward), there are two major categories: ratio schedules, in which food pellets are delivered to an animal depending on the number of responses the animal emits; and interval schedules, in which the animal must delay his response for a certain number of seconds before the reinforcer is available. Ratio schedules reinforce rapid responding; interval schedules reinforce precise time-based

responding. Ratio schedules are relatively impervious to weak environmental stimuli or drugs; interval schedules are sensitive to even very low doses of drugs. When a behavioral task involves the imposition of strong external stimuli on an animal, the animal is likely to pay attention to those task stimuli rather than to the effect of a weak environmental EM field. The principle has been elegantly demonstrated in a study of pigeons working on a fixed consecutive number schedule of reinforcement. When the animals were injected with methyl mercury their performance became variable and unstable. However, if a light cue was added to the task, indicating when the animal should shift to the reinforcement key, the animal's behavior became stable and appeared normal. If the light was removed, the animal's behavior immediately deteriorated again. Depending on the precise conditions of the task, the effects of methyl mercury were either easily discernible or completely hidden. The study has obvious implications for EM experiments, as well as for epidemiological studies. Workers intent upon performing a task may show no immediate evidence of the effect of an EM field, just as a soldier in battle may be wounded and not realize he was injured until the action ends.

Keeton (18) demonstrated a similar point in his study of the homing of pigeons. He strapped tiny magnets to their backs and observed their homing behavior. If it were a sunny day, the pigeons paid attention to the sun as a guide to their behavior and ignored the magnets. If it were cloudy, their flight was disoriented by the presence of the artificial magnetic field.

Even now, expensive studies are being funded by the United States government which use archaic, insensitive 19th century behavioral endpoints (foot withdrawal to shock, swimming endurance, open field tests, etc.). This is done in spite of the fact that a critical scrutiny of earlier studies presents compelling evidence that effects of weak EM fields could be reliably demonstrated if time-based schedules of reinforcement are used (15,16). This was especially shown in the work of Thomas and his associates (1-4).

CATEGORIES OF BEHAVIORAL MEASURES

Behavioral studies prior to 1980 have already been reviewed (15-17). In the following paragraphs literature from 1980-1985 will be considered (14). This follows the general format of the earlier reviews. Experiments will be grouped according to the type of behavioral measure used: activity, escape and avoidance, thermoregulatory, Soviet techniques, schedule controlled behavior, etc.

1. Activity Studies

The Argonne laboratories (19,20) reported that 60-Hz fields showed little effect on activity or circadian rhythms -- as one would expect. D'Andrea et al. (21,22) reported a failure to replicate a study from the Soviet Union in which exploratory behavior and catalepsy were the behavioral endpoints in a 50-Hz modulated 40-MHz field. Variable results were seen when locomotion was measured during long-term exposure to 915 MHz (5 mW/cm^2). As has been pointed out many times, none of these results are at all surprising since the behavioral measures are too variable to detect subtle effects.

2. Escape and Avoidance Studies

Escape and avoidance studies continue to show only marginal or variable impact of exposure to microwaves (23,24). Only intense fields (16 mW/g or greater) produce reliable escape responding (24,25). Again, these results are to be expected, since escape measures of behavior make heavy demands on the animals and are relatively insensitive to weak environmental stimuli. It is interesting to learn that escape and avoidance measures are adequate to detect effects of relatively high strength 60-Hz fields. Creim et al. (26) reported effects on the avoidance behavior of rats in high intensity 60-Hz fields (75 kV/m or greater). Hjereson et al. (27) reported corroborative results in rats exposed to 60-Hz fields of 90 kV/m or more. Swine appear to respond similarly to weaker (30 kV/m) fields when long durations of exposure are used (28). One study of weak magnetic fields reported no effect when passive-avoidance techniques were used and activity was measured (29). A novel study by Beel et al. (30) indicated that post-trial exposure to high levels of pulsed microwaves (18-22 mW/cm^2) can affect active or passive avoidance learning.

3. Thermoregulatory Studies

Thermoregulatory studies continue to be done, and continue

to be largely unenlightening. These studies demonstrate only that if microwave levels are high enough, the animals will be heated and can learn to emit behavioral responses to lower their environmental temperature (31-36). The authors' interpretations of these studies often go beyond the data and suggest that the demonstration of thermoregulatory behavior implies that there can be no direct effects of nonionizing radiation.

4. Teratogenic Studies

Teratogenic studies of behavior generally present weak evidence of the effects of high-strength fields (30 mW/cm^2) (37). Mitchell et al. (38) presented some evidence that endurance tests (swimming) may be affected by pre-natal exposure. Frey (39) found a variety of teratogenic effects following exposure to weak 60-Hz fields (3.5 kV/m). These studies suggest that 60-Hz fields may have more impact on teratogenic behavior than microwaves.

5. Other Measures of Behavior

Other measures of behavior that cannot be easily categorized in the present scheme have also been used. Frey and Wesler (40,41) presented evidence that conditioned emotional responses (CERs) and Sidman avoidance may be affected by low-intensity 60-Hz fields at 3.5 kV/m. Cooper et al. (42) indicated that conditioned suppression was affected by high level 60-Hz fields (50 kV/m) in pigeons. Clarke and Justesen (43) reported that a paradigm using Pavlovian operant conditioning was sensitive to the effects of 60-Hz and DC magnetic fields in chickens.

Microwave exposure affects certain dopamine and opiate related behaviors according to Frey and Wesler (44-47). Seaman et al. (48) indicated that some sexual behavior in rats was responsive to pulsed microwave fields.

6. Techniques Used in the Soviet Union

Techniques used in the Soviet Union for studying behavior continue to be used in the United States. Monahan (49) reported failure to replicate a Soviet study in which exploratory behavior and avoidance behavior were the endpoints. D'Andrea et al. (22) looked at open-field behavior, avoidance, and some unspecified operant behavior in a replication of Soviet studies of weak microwave effects (500 microwatts/cm^2, 2450 MHz).

Swim-to-exhaustion tests are reportedly enhanced by exposure to 15-kHz fields at 1 kV/m but not at 2 kV/m (50). Lobanova et al. (51) reported effects on conditioned reflexes of 10 mW/cm^2 microwaves, and dose-related changes as duration of exposure was increased.

7. Schedule-Controlled Studies

Schedule-controlled studies of behavior occupy a significant place among behavior experiments. My early work on both ELF and modulated VHF fields used time-based schedules of reinforcements with monkeys, neonatal chicks, and wild mallard ducklings (52-55). These experiments offered considerable promise for the sensitive and reliable detection of EM effects on behavior.

The work of Thomas and his associates (1-4) is remarkable for both its subtlety and reliability. It is distinguished by the use of time-based schedules of reinforcement, by the exploration of the interaction of EM fields with low doses of drugs, and by the use of pulsed, rather than CW, EM fields (56). He found that pulsed fields did not affect the dose-effect function of chlorpromazine or diazepam; nor did CW fields affect behavior modified by diazepam or chlordiazepoxide. Earlier results had shown that pulsed fields, however, did affect the response to chlordiazepoxide. These results imply (1) "...that drug class alone does not adequately predict outcome" and (2) that field parameters (CW or pulsed) are an important variable. In another study, dextroamphetamine and pulsed microwaves were shown to affect time-based schedules of reinforcements in rats (3). At 10 and 15 mW/cm^2, Thomas and Banvard (4) found that pulsed microwaves selectively lowered response rates on a time-based schedule of reinforcement, and that CW fields did not affect the response rates. Attempts by Lovely et al. and Lundstrom et al. (57-59) to supposedly replicate some of Thomas' work met with failure, probably because they were not replications due to differences in field exposure conditions (e.g., the use of different pulse repetition frequencies).

Gage (60) reported that CW microwaves did not affect d-amphetamine/microwave interactions when a complex mixed schedule of reinforcement was used. He did report however, that

length of exposure to 10 mW/cm^2 (2.0 W/kg) differentially affected a similar complex schedule (61).

Lebovitz (62-64) found that fixed-ratio responding in rats was not affected by microwaves more than was responding during time-out. He showed that externally-cued ratio-responding was less sensitive to microwaves than non-cued bar-pressing. Both findings corroborate our general understanding of schedule-controlled behavior and nonionizing radiation. Using a fixed-ratio/time-out schedule, Lebovitz could not detect any differences between pulsed and CW microwaves. However, some variation of a time-based schedule may have revealed such a difference. Lebovitz and Orr (65) found that the time-out portion of the fixed-ratio/time-out schedule was affected by CW microwaves, pulsed microwaves (3.5 mW/g), and low doses of phenobarbitol.

Extremely low frequency (ELF) modulation (3 Hz and 16 Hz) of EM fields (450 MHz) differentially affected fixed-time, schedule-controlled behavior of wild mallard ducklings (66). This study draws attention, again, to the significance of low-frequency modulation, and time-based schedules of reinforcement. It also suggests that species differences may be important and that migratory animals may be especially sensitive to EM effects, since neonatal chicks (54) did not show such a response.

Studies of the effect of ELF fields on schedule-controlled behavior by Feldstone et al. (67,68) have not yielded clear results. The research design appears to be overly complex. Stern et al. (69,70) reported that schedule-controlled behavior can be used to determine that the threshold for detection of 60-Hz fields generally lies between 4 and 10 kV/m for rats.

Finally, the study by Thomas, Schrot, and Liboff (5) is indeed one of the most dramatic of the 1980s. One can see that the significant variables in this study could be readily predicted from the existing data base (time-based schedules, low frequencies). In this study, rats were exposed to a 60-Hz field of 4×10^{-5} T rms, together with a static magnetic field of 2.61×10^{-5} T (half the geomagnetic field), and showed change in time-based schedules of behavior. The study has special interest because the 60-Hz frequency was chosen on the basis of the cyclotron resonance frequency of lithium ions.

PULSED OR MODULATED FIELDS vs CW FIELDS

Here one is looking not only for an effect, but for a differential effect. If the behavioral measure is not appropriate, a difference between pulsed and CW will not be observed. At present, the weight of evidence suggests that such a differential effect exists.

In behavioral studies of nonionizing radiation that were begun in 1966, Gavalas (Medici) examined the effect of low-frequency fields (7-75 Hz, 1-56 V/m)(71). Inter-response time schedules of reinforcement were performed by highly trained monkeys. These studies demonstrated that the animals' behavior was significantly modified (in the direction of shorter inter-response time). It was further shown that the animals were especially sensitive to the frequencies that were in the EEG range of the animals, that is 7 Hz, as contrasted with 45 Hz and 75 Hz. EEGs of the animals were analyzed and a change in the spectrum of the EEG was found when the animals were exposed to the nonionizing radiation.

In view of these results, Kaczmarcek, a young English neurochemist at UCLA, was asked to consider other ways to measure brain response to the fields. He initiated experiments with calcium efflux measurement following exposure to ELF fields. The studies on calcium efflux provided good concordance for the behavioral studies. Modulation was of key importance (72). Using modulated, 450 MHz fields, evidence was found for changes in calcium efflux from the in vitro brain of neonatal chicks. At the same time, a program of behavioral studies was begun, but not finished, in which effects with time-based schedules of reinforcement were to be compared using increasingly complex schedules.

Thus, there was evidence that in EM behavioral studies (1) the type of behavioral schedule used was very important; (2) the modulation frequency (the ELF frequency) of the field was very important; and (3) this frequency was relevant to what was going on neurophysiologically and neurochemically in the animal.

Unfortunately, those behavioral studies were not actively pursued. One of the major criticisms of the calcium efflux work, as it now stands, is that the observed neurochemical changes have not been linked experimentally to the behavior of the animal. The biological significance of the biochemical changes in the intact animal has not been adequately established.

The ELF modulation frequencies of the 450 MHz fields were selected on the basis of what was known about the EEG pattern of the monkey. This is a prime point that was lost on later researchers.

The importance of modulation can also be seen in the early work of Kalmijn (73) on detection of prey by sharks, which use passive electrosensing. He noted that it was important to simulate the ELF field produced by the breathing of the prey. The electrodes that he placed in the bottom of the shark's tank were not simply emitting DC fields but also contained a 4-Hz component to mimic the breathing of the prey. Again, the frequency was important and was particular to the organism and its ongoing activity.

In the years that followed, investigators were mindful of the possibly greater effect of pulsed vs CW fields. However, except for Frey and his experiments with brain-stem evoked responses (74), and heart responses (75), they looked at pulsed frequencies associated with common high-frequency field devices. None of the other investigators doing behavioral studies pursued the more precise idea of linking the modulation of the field to the exact ongoing physiological rhythms of the animal at the time of exposure.

Modulated vs CW fields in a variety of behavioral experiments will now be compared. Again, we will categorize these experiments according to the behavioral technique that was used.

In the 1970s some investigators, including Hunt et al. (76) found evidence for changes in activity in rats following exposure to pulsed microwaves. Servantie et al. (77) reported effects at intensities as low as 0.7 mW/cm^2. Other investigators such as Gage (78) and Roberti et al. (79) reported no effect on activity for CW fields. However, it is impossible to draw firm conclusions about the effect of pulsed vs CW fields in these studies because activity, as a measure, is so variable that real differences between the two field parameters may have been lost.

Studies of schedule-controlled behavior done in the 1970s revealed a mix of results. However, the studies of Thomas and

D'Andrea et al. (22) reported a study in which 50-Hz modulation was used in a 40 MHz field. However, behavioral measures, which were modeled after a Soviet study, were very crude. Effects on exploratory behavior and catalepsy were recorded. Not surprisingly, no effects were observed.

Seaman et al. (48) reported that low-frequency pulsing of microwave fields (10 pps, 3100 MHz) affected selected aspects of mating behavior in rats.

Other studies done in the 1980s have used pulsed fields or low-frequency fields, but the results appear to be variable and isolated. Feldstone et al. (67,68) did some experiments on the effect of 60 Hz on a variety of behavioral measures in the baboon. Beel et al. (30) have done a suggestive study on the effects of rather high levels of pulsed microwave following passive and active avoidance training in mice. Lai et al. (91) have reported that a variety of drug-induced effects are differentially influenced by pulsed microwaves.

In general, it may be concluded that modulation of microwave fields is more likely to affect behavior than CW fields, and this will appear if the behavioral test used is appropriate.

Studies using ELF fields have also shown effects on behavior. Frey (39) reported that rats exposed _in utero_ to 3.5 kV/m, 60-Hz fields showed effects in a variety of typical teratogenic measures such as acoustic startle, and surface righting. In a Sidman avoidance task, rats exposed to a similar field showed a diminished avoidance to the field which "...may indicate a decrease in timing capacity or reduced sensory response" (40).

Stern et al. (69,70) looked at behavioral detection of 60-Hz fields in rats and concluded that the threshold for direct detection lies between 4 and 10 kV/m. Earlier, Stern expressed concern that the detection behavior in his studies was confounded by other variables. More recently he indicated that it was not the case. Hjereson and his colleagues (27,28) found evidence that both rats and swine will avoid 60-Hz fields in a shuttlebox experiment. Studies from the Argonne Laboratory (19,20) with 60-Hz fields are flawed by the use of very simplistic behavioral measures. Cooper et al. (42) used a conditioned suppression paradigm to demonstrate detection of 60-Hz fields (50 kV/m). Clarke and Justesen (43) found increased variability in simple operant responding for food following Pavlovian conditioning in chickens that were exposed to DC or AC magnetic fields. The authors pointed out that the effects of the DC field might have been due to modulation of the field by the movement of the animals.

Finally, and most dramatically, Thomas et al. in 1984 exposed rats on a time-based schedule of reinforcement to weak 60-Hz magnetic fields and found marked changes in their behavior (5). Liboff, earlier, had calculated cyclotron resonances for lithium ions at 60 Hz. This experiment brings together sensitive behavioral measures (time-based) with biologically relevant frequencies. The hypotheses suggested by the research of the 1960s have finally been tested.

In summary, the weight of evidence suggests that the pulsing of nonionizing radiation and the use of ELF nonionizing radiation are extremely important factors in studies of behavior. Effects will not be found unless appropriate tests of behavior are used, such as time-based schedules of reinforcement. It is disappointing that so few studies have followed the lead of the research of the 1960s which indicated that even more dramatic effects would be seen if pulsing or modulation were done at very low frequencies. None of the noted studies, except the Thomas et al. study with 60-Hz magnetic fields (5), have considered ongoing physiological or biological rhythms in the animal.

No studies have yet looked at the impact of gradually increasing the depth of modulation as Czerski (personal communication) suggested in the early 1970s. More studies need to be done at low modulation frequencies and more studies need to be done to directly compare, as Lebovitz, Frey, and Thomas have done, the effects of pulsed and CW fields. It may be especially interesting to compare ELF fields and microwave fields that are modulated at ELF frequencies; e.g., 60-Hz ELF fields and microwave fields that are modulated at 60 Hz.

ELECTRIC vs MAGNETIC FIELDS

The Thomas et al. study (5) brings us to a consideration of what must now be considered a third major variable of significance for the study of the effects of EM fields on behavior. It seems clear that magnetic fields may have evolutionary and biological significance, at least for some animals. In those cases, one may expect that magnetic fields will show more influence on behavior than will electric fields. Direct comparisons of electric and magnetic fields have not yet been made. The dramatic experiments of Delgado have been described (92), and the interested reader is referred to his article on magnetic fields, brain, and behavior.

CONCLUSIONS

This review of behavioral studies indicates that there is clear, solid evidence that (1) time-based schedules of reinforcement repeatedly reveal effects of nonionizing radiation even when power levels are very low; (2) pulsed fields have more impact than CW fields; and (3) magnetic fields are particularly influential in some, and perhaps all, species.

Many very interesting studies remain to be done. Studies need to be done with complex modulation of the EM fields. Studies need to be done to explore CNS mediators of the behavioral effects that are observed. Conversely, behavioral studies need to be done to validate the efficacy of CNS theories about mediators. Frequency-specific studies that are appropriate to a given species and a given kind of behavior need to be done. Long-term studies need to be done to determine if cumulative effects exist.

An exciting array of studies can be pursued with the sophisticated behavioral techniques that are available to us. Simplistic and inappropriate behavioral studies did little to enlighten the research of the past and offer no hope for the future.

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23

Evolution and Results of Biological Research with Low-Intensity Nonionizing Radiation

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INTRODUCTION

Until the late 1940's, the primary interest in the biological effects of radiofrequency (RF) radiation was in heating for medical applications (1). Thus, the dominant theme of the research was the use of high powers to heat tissue. With the development and increasing use of RF radiation as radar in the 1940's, however, questions were increasingly raised about the possibility that exposure to this energy would have adverse biological effects on military personnel and workers.

In the mid-1950's, the Department of Defense's (DoD) RF hazards assessment establishment contracted for research to determine if there were adverse biological effects of RF radiation: the Tri-Service Program. The primary thrust of the program was essentially determined by the implicit assumption upon which prior work was based. It was assumed that the only way the energy could affect an organism was through overloading its heat-dissipation mechanism. Thus, little effort was expended to determine the effect of low-intensity energy. This assumption also resulted in an acrimonious dispute between those who contended that only thermal effects could occur and those who thought that nonthermal effects could also occur. But the fruitless argument was really the result of a semantic problem. The participants were talking past each other, for there never was a common definition of the words thermal and nonthermal. It was also assumed in the Tri-Service Program that nervous-system function and behavior could not be affected, so the possibility that modulation would be of consequence was essentially ignored (2). The Tri-Service Program was terminated in 1961, after gathering some data on overloading the temperature-regulating system.

Through the 1960's and early 1970's, there was some research on the biological effects of low-intensity RF energy. This was a distinct departure from the pattern of prior research. It was possible because the very limited funds available for the research were not controlled by those whose interests were in hazards or medical applications. This funding for research on low-intensity RF bioeffects continued through most of the 1970's.

Beginning in the early 1970's, a new program of research on high-intensity radiation effects, again primarily sponsored by the DoD's RF hazards establishment, was superimposed on and overshadowed the low-intensity research. By the late 1970's, the low-intensity research was being squeezed out because of the concentration of control of the funding into the hands of those in the DoD RF hazard establishment. The details on the control of research funds and its effects can be found elsewhere (1,3,4).

In the early 1960's, I initiated some of the early research in this country on bioeffects of exposure to low-intensity RF radiation. I was the most active investigator in this area during the 1960's and on into the 1970's. Thus, I have been given the task to trace chronologically, through my own research and the research of others in the US, the development in this country of biological research with low-intensity RF energy. The objective is not to give a comprehensive in-depth review of all aspects of RF biological research during those two decades and into the 1980's. Rather, the objective is to indicate the development of the more significant patterns of research, and to indicate where the research would likely lead if pursued as science. Since I was almost alone in doing research on low-intensity RF radiation bioeffects in this country during the 1960's, the beginning of this narrative will be primarily about my research.

THE QUIET DECADE

In the late 1950's, neurophysiological theory on information

transfer in the nervous system did not provide much understanding of neural function. It was about this time that I became curious about electric fields and the possibility of their interaction with the nervous system. In 1960, I was working at General Electric's Advanced Electronics Center at Cornell University doing biological research. One line of research I had initiated there was concerned with electrostatic fields and nervous-system function. I was also experimenting with air ionization and its biological effects. Late that year, while attending a small conference sponsored by the General Electric Company (GE), I happened to talk to a GE technician whose job was to measure RF radiation in the vicinity of radars. He mentioned that he "heard" radars. I found this to be interesting, since I, as well as everyone trained in the life sciences, had been taught that people hear acoustic energy and see, as light, electromagnetic energy. He was rather surprised when I asked if he would take me to a site and let me hear the radar. It seemed that I was the first person he had told about hearing radars who did not dismiss his statement out of hand.

A few weeks later, I went to the radar site and I heard the RF radiation. I performed a few simple tests to assure myself it was not an artifact. I then undertook a series of experiments which resulted in the publication of a brief article about the phenomenon in 1961 and a more detailed article in 1962 (5,6). I laid out the data from a variety of tests with humans. I suggested that there were probably multiple mechanisms for such an effect, but there was not sufficient data to specify mechanisms. Although the articles provoked interest in some members of the biological community and disbelief in others, there was little immediate activity by others to pursue the findings.

I searched the literature for information about the nature of RF field interaction with biological organisms and tissues. In essence, I found that there had been little effort in this country to consider sensory phenomena such as I was reporting, nor was there any significant research on neural effects of RF radiation. Virtually all of what little research existed had been done in the Soviet Union, but the translations were generally of poor quality and almost uninterpretable.

I expanded my literature search and published a comprehensive paper in 1965 (7). I assessed biological interactions with a wide portion of the electromagnetic spectrum, from the infrared down to low frequency. I critiqued the literature available, offered suggestions as to the portions of the spectrum with which the more significant research could be done, and pointed out the possibility of micron-wavelength emission from active nerves. This analytical review evoked a considerable amount of interest, for I received almost 5000 reprint requests.

After the initial exploratory work with the hearing phenomenon and concurrent with the preparation of the analytical review, I initiated further research with RF radiation. The series of experiments I carried out in the 1960's centered about four major themes: (1) experimental controls and techniques; (2) brain function and behavior; (3) sensory function; and (4) heart function.

Although the results of my work on experimental controls and techniques are too extensive to review in detail here, they are critical for accurate data collection. As a sampling, comparative studies of biological data recording techniques were done, including assessment of recording electrode systems in RF experiments. It was found that certain conventionally used systems yielded artifacts as data, due to induced currents stimulating the tissue as well as feeding into the recording preamplifier. It was found that filtering had limited usefulness and that lead placement was of consequence. New types of recording electrodes were developed which showed excellent characteristics in the RF field. In fact, the sponsor of one of these studies had the electrode patented (8,9).

Experiments were also carried out to develop techniques to remotely monitor the activity of nerves in an RF field. A method to record neural activity with no recording devices in the field was developed (10). Studies were made of restraint devices to hold animals, and of the RF field distorting effects of these devices. Polystyrene head holders were developed for use with cats. Teflon and nylon chairs and restraints were developed in studies with monkeys, and wooden enclosures and restraints were developed for use with cats (11,12).

Experiments were carried out using three-dimensional field plots to investigate the effect of the biological object itself on the field within an RF anechoic enclosure. Similar studies were made on the perturbing effect of field measurement devices on the field. Standardized methods of measurement and reporting of measurements were developed. Experimentation was also carried out to determine the effect of body position and its orientation on results. Studies were made of shielding materials and their usefulness in experimentation (11). I found in my other experiments that carrier frequency and modulation had to be controlled because they were critical in the effect of low-power-density RF radiation on some functions of higher organisms (6,11,13-15).

As may be seen from this sampling, there are many variables that need to be controlled and special techniques that must be used in biological work with RF radiation. But the literature shows that many of these variables have not been controlled.

Turning now to the second theme, data specific to brain function, I shall summarize the information obtained. Cats were illuminated with pulse-modulated RF radiation and evoked activity in the brain was observed (11). The threshold average power density necessary to evoke activity was approximately 20 microwatts/cm^2. The controls used indicated that the activity was neural evoked activity rather than an artifact of the situation. Using an Echosorb shield to cover the entire cat, or head, or body, it was found that the head must be exposed to the radiation in order to have an effect occur. Within the carrier frequency range used, there appeared to be a reduction of effect at the highest frequency. Variation in power density had a distinct effect on the evoked activity. Polarization of the energy, whether perpendicular or parallel to the spine, did not seem to matter. As pulse repetition frequency (PRF) was changed, the evoked activity did not change significantly until the PRF was greater than approximately 50 pulses per second (pps). In general, recording from the rostral brain stem did not yield evoked activity as diffuse and persistent as recording from the caudal portion of the reticular formation of the brain.

In view of what I was seeing in using RF radiation to influence neural tissue, and because of ideas I had about neurophysiological theory, I expanded the brain function experimentation to assess the possibility that nerves, when active, would emit coherent electromagnetic energy. It seemed that the channel capacity indicated by neurophysiological theory was insufficient to encompass the results of many neurophysiological and behavioral experiments, and that there might be communication between nerves via the emission of electromagnetic energy at micron wavelengths. To assess this possibility, I set up an experiment using some of the equipment I developed for remote sensing. I used live nerves from the legs of blue crabs because of their characteristics. I sought to determine whether there was emission of micron wavelength energy when the nerves were active. I found that the emission was considerably greater than what would be expected from a black-body nerve model. I established that the emission was not an artifact, that the emission was from the surface of the nerve, and calculated the amount of emission and its spectral band (10). A number of subsequent papers by others used these findings in their development, at the molecular level, of new conceptualizations of neural function. These include Lee's concepts on the role of excitons and phonons on nerve permeability and propagation of impulses (16), Cope's micron-wavelength concepts on phonon coupling and IR involvement in nerve (17), and Maurel and Galzigna's (18) definition of the involvement of the dipole moment of acetylcholine in neural chemical transmission. There are other similar papers relevant to low-intensity RF radiation bioeffects (19-32).

The third theme was an extension of the RF hearing research and an exploration for other sensory effects. No visual effects were found at that time, but tactile stimulation in humans at very-low frequency (VLF) carrier frequencies was found (13,14). An attempt was made to determine the locus of the RF hearing mechanism. I searched for cochlear microphonics in guinea pigs and cats exposed to RF radiation, but found none(13,14). The in-air RF hearing thresholds for humans were determined for two carrier frequencies. Since they were quite different, a mathematical model of layers of head tissue was constructed. As RF energy passes through each layer of tissue, the absorption of the energy differs as a function of carrier frequency. Thus, I sought to determine mathematically where in the head the RF energy from the two frequencies became equal. Such an equality point, the crossing of signal strengths, would suggest where to

look for the sensing mechanism. In constructing the model, all tissue electrical values were selected in advance, standard values for tissue thickness were used, and first reflections were taken into consideration. The calculations indicated that the RF energy crossing was in the fluid at the first bone/soft-tissue interface. This suggested a locus in the cochlea or at the surface of the cerebral cortex.

Experimentation was also carried out with cats, using the avoidance conditioning technique to determine if they could sense RF energy. Cats avoided the radiation and thresholds were established. In experiments with rhesus monkeys, avoidance behavior also appeared.

The last major theme of my 1960's experimentation concerned heart function. The isolated frog heart, stripped of its neural and hormonal buffer systems, was exposed to RF radiation (15). It was found that the heart was responsive to RF radiation when the pulses were synchronized with certain phases of the heart cycle. When the RF pulse occurred about the time the QRS complex occurred, the beat rate increased. In half the cases, arrhythmias occurred, and occasionally the heart ceased beating after a period of arrhythmia. No such effect appeared when the heart was illuminated at earlier points in the cycle.

During the 1960's, others also reported on experiments with low-intensity RF radiation. For example, Hearn (33) explored the effect of long continued low-intensity RF energy on visual acuity. He found significant differences in the flicker thresholds of irradiated as compared to nonirradiated subjects. Korbel and Thompson (34) exposed rats to what they believed to be low-intensity RF energy. They found that irradiated subjects were more active than nonirradiated subjects for a short period of time during the early part of the experiment, but they became less active than the nonirradiated subjects as the days of radiation exposure increased. In a follow-up study, Korbel and Fine (35) explored a possible relationship between RF frequency range and activity level, but they had equipment problems that left their results in doubt. Bourgeois (36) found that exposure to RF radiation resulted in a significant decrease in auditory thresholds in humans. The threshold change was found to be a function of the type of modulation used, since auditory thresholds were significantly lower upon exposure to 1000-Hz modulated RF radiation than upon exposure to 300-Hz modulated RF radiation.

The foregoing summarizes the primary lines of biological research with low-intensity RF radiation in this country during the 1960's. I had spent most of the decade laying a foundation in data for the study of RF radiation interaction with biological organisms and tissues. Although I would get reprint requests in the thousands for some of my reports on experiments, it was a rather quiet and lonely effort that was, however, quite interesting.

THE LIVELY DECADE OF THE 1970's AND INTO THE 1980's

INTRODUCTION

The period of my quietly doing research came to an end in 1969 with the passage of Public Law 90-602, the Radiation Control for Health and Safety Act. The purpose of the law was to protect the public health and safety "...from the dangers of electronic product radiation." The Bureau of Radiological Health, Department of Health, Education and Welfare, became active in the area because of the law. The hazards people of the DoD, who had been involved in the Tri-Service Program, again became active in the area.

The Bureau convened a symposium in September of 1969 in Richmond, Virginia, that I helped organize. The topic of the symposium was "Biological Effects and Health Implications of Microwave Radiation." I presented a paper entitled "Effects of Microwaves and Radio Frequency Energy on the Central Nervous System" (37). In it, I detailed why there was so much misunderstanding and confusion in the area, and summarized some of my research. I spelled out lines of research I considered to be worth pursuing, techniques that could be used, and the controls that had to be used in order to get valid data. During the next few years, I found myself spending a large proportion of my time answering phone calls and letters from scientists. DoD had started funding research in the area. The world was not so lonely any more.

MECHANISMS

The decade of the 1970's opened for me with the preparation of a paper in which I presented some of my thinking on possible mediators or mechanisms for biological effects of very low-intensity RF radiation (2). It is my nature to look at the broad picture and to integrate. Most of my experimentation is done because I have reached a choice point in my theorizing. In order to decide which way my thinking should go, I do an experiment to provide data for the choice. This is why I do such a diversity of experiments.

In the preparation of that paper, I made explicit some of my thinking (2). Much of what I said then is still relevant, for much of the research that was done during the 1970's was irrelevant to the questions about the biological effects of low-intensity RF radiation. The DoD sponsors who determined what would be done appear to have been primarily interested in research that used high power levels or used techniques relevant to thermoregulation questions.

In that paper, I identified the mistaken assumptions that formed the basis of Schwan's notions about nervous-system function. Those notions had inhibited research on low-intensity and nervous-system effects since the 1940's. He had set up a mathematical model of the axon membrane, and assumed that it was a reasonable representation of the nervous system (38). His calculations with the model indicated that at field strengths that are "not thermally significant," the induced potentials across the nerve membrane are many orders of magnitude smaller than the nerve resting potential. He stated that such induced fields applied to the resting potential of the axon cannot excite the nerves, and essentially, on the basis of this, he concluded that the nervous system could not be influenced by low-intensity RF radiation.

I pointed out that there were at least two faults in his reasoning. One was that his implied model of the nervous system was unrealistic. Nerves function, and the resting potential is only one extreme of a continuum of potentials on the axon. He ignored most of the nerve cell, including the most important part, when he considered only the axon in his model. Further, nerves interact, and the points of interaction on the cell bodies are the most sensitive to disturbance, not the axon.